The expression pattern of *WRKY10* gene under salt stress conditions in two contrasting (*Triticum aestivum* L.) wheat genotypes

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Salinity is one of the most serious environmental factors that limits the productivity of agricultural crops worldwide. Majority of the plant responses to stressors are controlled at the transcription level, in the presence of transcription factors, in particular, those that belong to the WRKY family. In the present study, expression profile of the *TaWRKY10* gene was examined using RT-PCR method under salinity in contrasting wheat genotypes. The *TaWRKY10* gene activity was found to be different in tolerant (Saratovskaya 29) and sensitive (Gyrmyzygul) genotypes under salt stress. The expression level of the WRKY10 gene was found to be higher in the tolerant genotypes compared with the sensitive genotypes under salt stress of various duration. The obtained results and literature data suggest that the WRKY10 gene is involved in some signal pathways playing a significant role in responses of bread wheat to salt stress.

Keywords: *Salt stress, wheat genotypes, gene expression*

INTRODUCTION

Plants under natural climatic conditions are exposed to effects of abiotic as well as biotic factors throughout their lifespan. Stresses have direct, negative effects on the biochemical and physiological processes that are associated with plant growth and development, which results in a significant reduction in crop yield. Therefore, mobilization all the existing adaptive mechanisms is required for maintaining plant viability under unfavorable conditions. Complex mechanisms of the response to external stress signals were formed in plants during the evolution process and these mechanisms of tolerance and resistance are mainly controlled by the net of the structural genes, regulated by the transcription factors (TFs) (Zhu, 2004). Transcription factors are protein complexes that bind to specific *cis*-acting promoter elements thereby activating or repressing the transcriptional rates of their target genes (Rajarshi and Pradeer, 2013). Transcription factors occupy a significant volume in the genome and can be divided into more than 50 families. In the review by Wang and collaborators (Wang et al., 2016) numerous examples of transgenic plants overexpressing various transcription factors and expressing higher resistance to abiotic stresses have been presented. Based on the content of specific domains, transcription factors are divided into various families.

WRKY TFs are one of the largest families of transcriptional regulators in plants and are characterized by the presence of one or two 60-amino-acid WRKY domains (Eulgem et al., 2000; Rushton et al., 2010). A common feature of the WRKY domain is the highly conserved WRKYGQK heptapeptide at its N- terminus and a zinc finger-like motif at its C-terminus. It is generally assumed that the WRKY domain can activate or repress the transcription of target genes by specific binding to various W-box elements with an invariant GAC core sequence present in the promoters (Brand et al., 2013).

Based on the number of WRKY domains and the type of zinc-finger motifs, WRKY proteins have been classified into three main groups (I, II and III). Group I typically contains two WRKY

domains, including a C_2H_2zinc-finger structure. Groups II and III are characterized by a single WRKY domain, including a C_2H_2 and C_2HC zinc-finger motif, respectively. In addition, group II was subdivided into five subgroups, (IIa-IIe), based on phylogenetic analyses (Euglem et al., 2000; Rushton et al., 2010; Rinerson et al., 2015). Members of the WRKY family regulate gene expression by exclusively binding to the W-box (TTGACC/T), which is a cis-element in the promoter region of target genes (Bakshi and Oelmüller, 2014; Ulker and Somssich, 2004).WRKY are represented in various plant species, and for each of them significant variations of features are characteristic.For instance, there are 109, 72 and more than 171 WRKY family members identified in rice (Eulgem and Somssich, 2007), arabidopsis (Zhang and Wang, 2005) and wheat (Ning et al., 2017), respectively.

Accumulating evidence has demonstrated that WRKY proteins are involved in multiple aspects of plant growth, development, and stress response. In recent years, an increasing number of WRKY TFs has been found to be involved in plant responses to abiotic stresses. For example, in soybean (Glycine max), 25 out of 64 tested WRKY genes respond to high salinity, drought or cold treatments (Zhou et al., 2008; Song et al., 2016), and comparable results have been reported in Arabidopsis (Jiang and Deyholos, 2006), *Gossypium aridum* (Fan et al., 2015), wheat (*Triticum aestivum* L.), rice, etc. (Berri et al., 2009; Niu et al., 2012).

Wheat (*Triticum aestivum* L.) is one of the four major cereals in the world. As one of the most important agricultural crops, wheat is a staple food crop for a large portion of the world's population. Unfortunately, its production is severely affected by adverse environmental stresses. Soil salinity is one of the main limiting factors, negatively affecting growth and development of wheat. Grain quality deteriorates and grain yield declines under salinity. Salt stress is a genetically complex trait, often modulated by multiple biosynthetic and signaling pathways. Some wheat genotypes possess a unique ability to rapidly adapt to salt stress, whereas others are highly sensitive because of their genetic makeup and regulatory architecture. Under stress, the expression of various genes, the products of which are necessary for the plant to survive adverse conditions, is induced. Therefore, the identification and functional study of stress responsive genes will elucidate the molecular mechanisms of the plant stress response and tolerance, and will ultimately lead to improvement of stress tolerance in wheat. Identification of expression profiling of TFs plays a crucial role to understand the response of different wheat cultivars against severe environmental changes. In this study, the effects of salinity stress on expression pattern of TaWRKY10 TF gene were investigated in two *T.aestivum* genotypes differing in salt tolerance.

MATERIALS AND METHODS

Growth of Plants and Stress Applications

The seedlings of salt-tolerant (Saratovskaya-29) and salt sensitive (Gyrmyzygul) bread wheat genotypes were used in this investigation. Seeds were surface sterilized with 10% NaOCl solution, rinsed with distilled water, thereafter they were sown on moistened filter papers placed in Petri dishes. Germinated seedlings were transferred to a nutrient solution and plants were grown until 10 days old, thereafter the seedlings were subjected to salinity stress. Sodium chloride (NaCl) was added to the nutrient solution to obtain 150 mM NaCl. Shoot samples from salt-treated and control plants were harvested after 24 and 48 h of stress application and immediately frozen in liquid nitrogenand stored at -80°C until further use. Time point zero (0 h)was used as a control. Each set of the experiment was repeated three times and samples from each set were used as biological replicates.

RNA Extraction

Total RNA from the leaf tissues of salt-treated and untreated Saratovskaya-29 and Gyrmyzygul genotypes was extracted by using the TRIzol reagent (Invitrogen) according to the user manual. All RNA samples were treated with of DNase I enzyme (MBI Fermentas, Hanover, USA) at 37°C for 1 h. to remove any contaminating genomic DNA before cDNA synthesis. RNA was extracted with phenol and precipitated in ethanol. To determine RNA

concentrations, optical density of the preparations was measured at 260 nm using a spectrophotometer BioTek Epoch. The RNA integrity was tested by electrophoresis on 1% agarose gels.

RT-PCR analysis

The first strand cDNA was synthesized using 2μg total RNA in a 20 μL reaction mixture containing 40 pmol oligo $(dT)_{18}$ primer, 1× reaction buffer with 10 mmol/L dNTP mix and 200 U of Moloney Murine Leukemia virus reverse transcriptase enzyme (MBI Fermentas, Hanover, USA). The reaction mixture was incubated for 1 hour at 42°C, followed by heating for 10 minutes at 70°C. The first cDNA template was diluted 6-fold and kept at –20 °C for RT-PCR amplification analysis.The first-strand cDNA generated from total RNA including salt-treated and untreated samples from either the Saratovskaya-29 or Gyrmyzygul genotype was subjected to reverse transcription PCR analysis. To examine the expression of TaWRKY10, semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out using the gene-specific primers (Table 1). The PCR was performed in a 20 μL reaction mixture at an annealing temperature of 63°C with 35 cycles. Actin gene of *T. aestivum* was used as a reference gene. The amplified products were separated by 1.2% (w/v) agarose gel electrophoresis. The band intensity was visualized and photographed by a gel documentation system («UVIPRO», UK). To ensure the reproducibility of the results, the experiment was repeated three times.

RESULTS AND DISCUSSION

The first systematic identification and expression analysis of 15 wheat WRKY genes, which were cloned from *T. aestivum* L., were reported in 2008 (Wu et al., 2008). A total of 8WRKY genes were responsive to low temperature, high temperature, NaCl or PEG treatment.

Niu et al. (2012) characterized forty-three putative TaWRKY genes from *T. aestivum*. TaWRKY2 and TaWRKY19 genes were induced upon treatments with drought and salt stresses. In the different study, 10 WRKY genes from genome of wheat (*T. aestivum* L.) were identified and cloned (Wang et al., 2013). Upregulation of TaWRKY10 was observed after treatment with polyethylene glycol, NaCl, cold and H_2O_2.

In our work the expression pattern of TaWRKY10 in response to salt stress, was analyzed in leaves of the Gyrmyzygul and Saratovskaya-29 genotypes of bread wheat, shown to be sensitive and tolerant to high salt, respectively. The complete cds *TaWRKY10* mRNA is 792 bp in length (GenBank accession no. EF368361.1), including a complete ORF of 672 bp that encodes a putative protein of 223 amino acids (predicted relative molecular mass of 24.5 kDa).

The *TaWRKY10* gene expression profile in wheat shoot tissues was studied using RT-PCR based on the content of transcripts, fixed on electrophoregramms. For this purpose, RNA was isolated from the leaves of the control and stress-exposed variants of the studied plants (Fig 1).Then cDNA was synthesized and PCR was performed with primers specific to the *TaWRKY10* gene. Sequences of the primers were taken from the article by Wu and collaborators (Wu et al., 2008). Performing PCR with the given primers, cDNA fragments of the expected size (about 800 bp) corresponding to the *TaWRKY10* gene were amplified (Fig. 2).

Comparison of the amplified cDNA fragments obtained on mRNA of the control and stress-exposed (150 mM NaCl) seedlings showed that the transcript level of the *TaWRKY10* gene pronouncedly increased in the leaves of the tolerant genotype Saratovskaya-29 as a result of the 24-h exposure to stress. During long-term NaCl exposure (48 h), the expression remained at the achieved level. A different pattern was observed in the sensitive genotype Gyrmyzygul.

After 24-h exposure to salt stress a slight increase in the transcript level of *TaWRKY10* occurred. Then after 48-h NaCl exposure the gene expression decreased. This means that the TaWRKY10 gene was expressed less actively in the sensitive variety than in the tolerant one under all regimes. This suggests that TaWRKY10 can participate in signaling pathways associated with increased tolerance of wheat to salinity.

Table 1. Primers for RT-PCR

Gen	Sequence (5′–3′)	Application	Product size (bp)
TaWRKY10	AGCTCGTCTGTGCAGTGCACTTAT TCGTGTACATGCATCCGTGAGATT	Full length amplification	800
TaActin	CTTGTATGCCAGCGGTCGAACA CTCATAATCAAGGGCCACGTA	Internal control	250

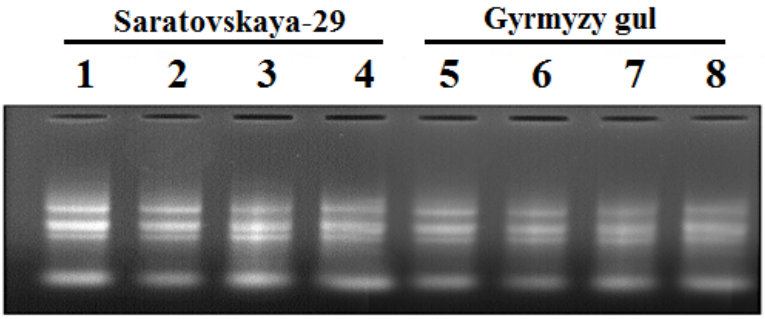

Fig. 1. Agarose gel electrophoresis of total RNA isolation from untreated and NaCl treated shoot tissues of 2 bread wheat genotypes; lanes 1-4 and 5-8 represent control plants (24 h), 150 mM NaCl (24 h), control plants (48 h) and 150 mM NaCl (48 h), respectively.

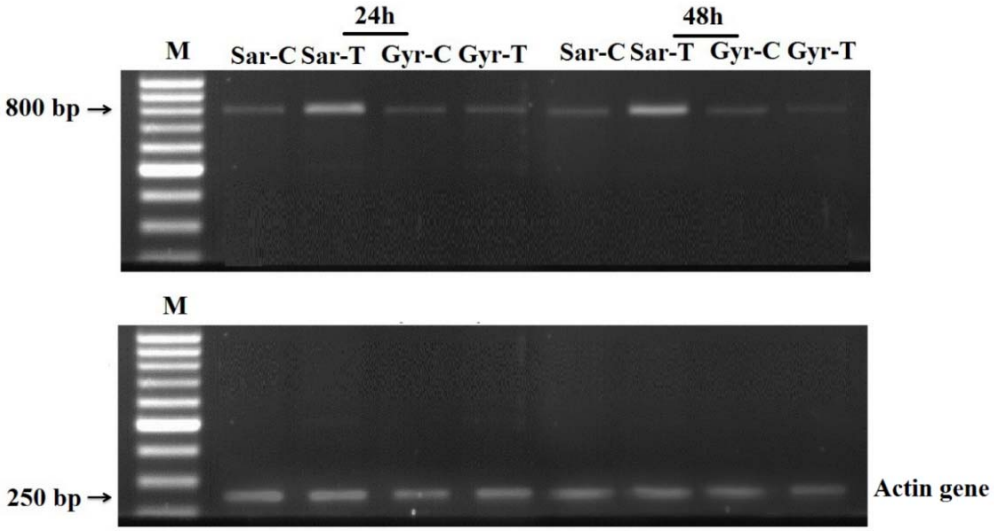

Fig. 2. Semi-quantitative expression analysis of *TaWRKY 10* gene (performed through reverse transcription-polymerase chain reaction) in shoots of the contrasting bread wheat genotypes Saratovskaya- 29 (Sar, salt tolerant) and Gyrmyzygul (Gyr salt sensitive) under control (C) and 150 mM NaCl stress (T) conditions.
Actin was used as a reference gene. Lane M: molecular weight marker (100–1000 bp).

Thus, in these experiments, marked genotypic differences were detected between the tolerant (Saratovskaya 29) and sensitive (Gyrmyzygul) varieties in the activity of the TaWRKY10 gene under salt stress.

According to previous investigations, expression of abiotic stress-inducible WRKY genes in various plants increased in the tolerant and decreased in the sensitive varieties under salt stress.

Because,TdWRKY expression in response to salt stress was found to display distinctive patterns in two durum wheat genotypes with contrasting behavior regarding tolerance to abiotic stresses. In the tolerant GR variety, TdWRKYs were strongly induced by salt stress within a few hours (6 h), while it was down regulated in the sensitive OR variety. Notably, differences between tolerant and sensitive genotypes were detected, mainly in the expression levels in tolerant genotype leaves at 24 h stress treatment (Yousfi et al.). Besides Penget al. (2014) showed that WRKY members' unigenes were mostly up-regulated under salt stress in cotton (*Gossypium hirsutum* L.). They noted that some WRKY genes were expressed in the salt-tolerant genotype Earlistaple 7, but were repressed, weakly induced, or not induced at all in salt-sensitive Nan Dan Ba Di Da Hua, within 24 h. HvvWRKY2 was induced by salt stress in TR1 (Tolerant variety) but not in TS1 (Sensitive variety) (Li et al., 2014).

It should be noted that in the literature known to us, data on the dynamics of expression of the TaWRKY10 during long-term (diel) exposure to stress factors, including salinity, are absent.

Further compiling of data on the study and comparative analysis of gene expression from different families of transcription factors in varieties with contrasting salt tolerance can serve as a basis for studying the key mechanisms of the regulation of gene expression under salt stress.

In addition, such a database can be used to identify the main trends and characteristics of salt tolerant varieties for the subsequent creation of the molecular markers for salt tolerance.

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Duz stresi şəraitində davamlılığına görə fərqlənən iki buğda genotipində (*Triticum aestivum* L.) *WRKY10* geninin ekspressiya modeli

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Şoranlıq dünya miqyasında təsərrüfat əhəmiyyətli bitkilərin məhsuldarlığını məhdudlaşdıran çox ciddi ekoloji amildir. Bitkilərin stressorlara qarşı reaksiyalarının əksəriyyəti, transkripsiya faktorlarının (TF), o cümlədən də, WRKY TF ailəsi üzvlərinin iştirakı ilə transkripsiya səviyyəsində idarə olunur. Təqdim olunan işdə duz stresinə məruz qalmış buğda genotiplərində WRKY10 geninin ekspressiyası RT-PCR üsulu ilə araşdırılmışdır. Duz stresi şəraitində tolerant (Saratovskaya 29) və həssas genotiplərdə (Gırmızıgül) WRKY10 geninin fəallığında müxtəliflik müşahidə olunmuşdur. Duz stresinin müxtəlif təsir müddətində davamlı genotipdə WRKY10 geninin ekspressiya səviyyəsinin həssas genotiplə müqayisədə yüksək olması müəyyən olunmuşdur. Alınan nəticələrə və ədəbiyyat məlumatlarına əsasən belə qənaətə qəlmək olar ki, WRKY 10 geni yumşaq buğda bitkisinin duz stresinə reaksiyasında mühüm rol oynayan bəzi siqnal yollarında iştirak edir.

Açar sözlər: *Duz stresi, buğda genotipləri, gen ekspressiyası*